

**Proteoglycan Compositions for the Treatment of Cardiovascular Inflammatory Diseases**

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**BACKGROUND OF THE INVENTION**

10 This application is a divisional/CIP of copending PCT/US02/00470, filed 01/03/2002, which is a CIP of copending US 09/771669, filed 01/30/2001m which is a divisional of US 09/056707, filed 04/08/1998.

15 [001] The invention is generally related to the treatment of inflammatory conditions. More specifically, the invention is related to compositions containing inhibitors of mast cell activation and secretion such as a proteoglycan that are designed to be used as dietary supplements or adjuvants to conventional approved medications for the relief of cardiovascular inflammatory conditions.

20 [002] There have been a number of mostly anecdotal reports that the proteoglycan chondroitin sulfate, as well as glucosamine sulfate, a product of the intestinal breakdown of proteoglycans, may be helpful in relieving the pain of osteoarthritis: - Shute N. Aching for an arthritis cure. *US News and World Report*, Feb. 10, 1997.- Cowley G. The arthritis cure? *Newsweek*, Feb. 17, 1997; Foreman J., People, and their pets, tout arthritis remedy. *The Boston Globe*, April 7, 1997; Tye L. Treatment gains scientific attention. *The Boston Globe*, Sep. 25, 2000.

25 [003] A recent meta-analysis showed potential therapeutic benefit of chondroitin sulfate and/or glucosamine in osteoarthritis [McAlindon et al. *J Am Med Assn.* 283:1469 (2000)], while a double-blind clinical trial with glucosamine showed definite benefits in osteoarthritis with respect to both pain and radiographic joint appearance [Reginster et al., *Lancet* 337:252 (2001)]. However, less than 5% of the chondroitin sulfate in commercially

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available preparations is absorbed orally, because the size of the molecule and the degree of sulfation impede its absorption from the gastrointestinal tract. Furthermore, such commercial preparations use chondroitin sulfate obtained from cow trachea, with the possible danger of contracting spongiform encephalopathy or "mad cow disease". In fact, the European Union has banned even cosmetics that contain bovine-derived products.

[004] Theoharides *et al.* *British Journal of Pharmacology* 131:1039 (2000) indicated for the first time how proteoglycans such as chondroitin sulfate may work. The paper reported that chondroitin sulfate and, to a lesser degree, glucosamine sulfate, inhibit activation of mast cells that are known to trigger allergy and asthma. This discovery is the basis for Theoharides, United States patent applications Serial No. 09/056,707, filed April 8, 1998 and 09/773,576, filed February 2, 2001.

[005] Mast cells are also now recognized as important causative intermediary in many painful inflammatory conditions [Galli, *N Eng J Med.* 328:257 (1993); Theoharides, *Int J Tissue Reactions* 18:1 (1996)], such as interstitial cystitis and irritable bowel syndrome [Theoharides, *Ann NY Acad, Sci.* 840:619 (1998)], as well as in migraines and possibly multiple sclerosis [Theoharides, *Persp Biol Med.* 26:672 (1983); Theoharides, *Life Sci* 46:607 (1996)]. In fact, glucosamine was recently considered to be prophylactic for migraines [Russell, *Med Hypoth* 55:195 (2000)].

[006] Mast cells are increasingly implicated in conditions involving inflamed joints, such as in osteoarthritis and rheumatoid arthritis, through activation of local mast cells by, for example, neuropeptides, such as Substance P. Additional indirect evidence also supports the involvement of mast cells in bone resorption: (a) systemic mastocytosis is invariably associated with osteoporosis; (b) inhibition of mast cell mediator release reversed lytic bone changes; (c) depletion of mast cells inhibited bone resorption in organ culture; (d) human synovial mast cells were shown to secrete in response to allergic

and non-immunologic stimuli; (e) human mast cells release the cytokine IL-6 and (f) IL-6 has been definitively linked to bone resorption and osteoporosis.

[007] It was recently shown that chondroitin sulfate's ability to inhibit the activation of mast cells compliments the inhibitory effects on mast cell activation of another class of naturally occurring compounds, the flavonoids [Middleton *et al. Pharm Rev* 52:1 (2000)]. Certain plant flavones (in citrus fruit pulp, seeds, sea weed) are now recognized as anti-allergic, anti-inflammatory, anti-oxidant and cytoprotective with possible anti-cancer properties. Only some flavonoids that belong to the subclass of flavones, e.g., quercetin, inhibit mast cell activation.

[008] Quercetin inhibits secretion from human activated mast cells [Kimata *et al. Allergy* 30:501(2000)], and has also been used effectively for the treatment of chronic prostatitis [Shoskes *et al., Urology* 54:960 (1999)]. However, other flavonoids may have opposite effects. Use of the term "bioflavonoids" or "citrus flavonoids" in certain commercial products, therefore, provides little information, and may include molecules that have detrimental effects; for example, soy contains isoflavones that have estrogen-like activity that worsens inflammatory conditions.

[009] Copending United States patent applications Serial Nos. 09/056,707, 09/771,669, 09/773,576, and 10/439,301 claim the oral and topical use of proteoglycans, without and with flavonoids, for the treatment of mast cell activation-induced diseases. Absorption of these compositions from the gastrointestinal tract and synergism with other treatment modalities were addressed in these applications.

[010] Applicant has described the use of antagonists of the action of Corticotropin Releasing Hormone (also known as Corticotropin Releasing Factor) in inhibiting myocardial mast cell activation in myocardial ischemia (copending United States patent application Serial No. 08/858,136, filed

05/18/97), in treating stress-induced skin disease (United States Patent No. 6,020,305) and stress-induced migraine headaches (United States Patent No. 5,855,884), the contents of which are incorporated herein by reference.

5 [011] Histamine-3 receptor agonists as pharmaceutical agents in mast cell-involved diseases are described in Theoharides United States Patent No. 5,831,259. The contents of these three patents are incorporated herein by reference. At the time of this invention the synergistic effects of the present compositions with such antagonists had not yet been recognized.

10 [012] An important need therefore exists for compositions for administration to human patients being treated for mast cell-induced cardiovascular inflammatory diseases by various modalities, that are synergistic in that they have stronger effects than the sum of the effects of the individual  
15 components, and also synergistic with conventional clinical treatments of inflammatory conditions. "Synergistic" is also intended to mean: "coordinated or correlated action by two or more structures or drugs" [Stedman's Medical Dictionary, 23rd edition, Williams & Wilkins, Baltimore, 1976]. An important need also exists for formulations that increase the  
20 absorption from the gastrointestinal tract, nasal passages and skin surface of the compositions of the invention. Such formulations have been discovered, and are described below.

## 25 SUMMARY OF THE INVENTION

30 [013] The invention comprises compositions for human use containing a sulfated proteoglycan and an unrefined olive kernel (pit) oil (hereinafter referred to as olive kernel extract ("OKE")), and one or more active ingredients selected from the group consisting of a sulfated hexosamine, a flavonoid compound, S-adenosylmethionine ("SAM"), histamine-1 receptor antagonists, histamine-3 receptor agonists, antagonists of the actions of CRH, caffeine, folic acid, rutin, bitter willow bark extract, hyaluronate salts such as sodium

hyaluronate, polyunsaturated fatty acids such as eicosapentenoic acids, niacin, polyamines, a chemotherapeutic agent such as tamoxifen, an interferon, together with appropriate excipients and carriers, said compositions having improved absorption from the gastrointestinal tract, skin surface, and nasal and pulmonary surfaces, and anti-inflammatory effects synergistic with each other and synergistic with available conventional clinical treatment modalities.

[014] In one embodiment, the sulfated glucosamine is D-glucosamine sulfate, the proteoglycan is non-bovine chondroitin sulfate, and the flavone is quercetin.

[015] In an other embodiment, compositions may also contain antagonists of the effects of CRH on mast cells or other target cells of the myocardium, gastric mucosa, urinary bladder, skin, meningeal membranes, and blood-brain barrier.

[016] In still another embodiment, the present compositions are used against superficial vasodilator flush syndromes.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[017] It has been discovered that a combination of a sulfated proteoglycan and a unique, unrefined olive kernel extract, together with one or more of a sulfated D-hexoseamine, a flavone, an CRH antagonist, a histamine-1 receptor antagonist, a histamine-3 receptor agonist, a rutin, a polyunsaturated fatty acid such as eicosapentenoic acid, niacin, polyamines, caffeine, a chemotherapeutic agent such as tamoxifen, an interferon, has synergistic anti-inflammatory effects when used as a dietary supplement, a topical product or an aerosol for nasal or pulmonary administration, without or with a conventional clinical treatment for inflammatory diseases. Such inflammatory diseases result from the activation, degranulation and consequent secretion

of vasoactive and inflammatory biochemicals from mast cells, and the resultant inflammatory diseases include the group consisting of: allergic inflammation, arthritis (to include osteoarthritis and rheumatoid arthritis), cancer, cardiovascular diseases, fibromyalgia, inflammatory bowel disease, interstitial cystitis, irritable bowel syndrome, migraines, angina, chronic prostatitis, eczema, multiple sclerosis, psoriasis, sun burn, periodontal disease of the gums, superficial vasodilator (flush) syndromes due, for example, to niacin ingestion, and hormonally-dependent cancers.

[018] Other natural sulfated proteoglycans suitable for practicing this invention include keratan sulfate, dermatan sulfate and hyaluronic acid salts, e.g., sodium hyaluronate. The preferred biological source of the chondroitin sulfate is shark cartilage which is more-highly sulfated than the common commercial chondroitin sulfate isolated from cow trachea; the shark cartilage source also avoids the potential dangers associated with bovine sources.

[019] The highly preferred flavone is quercetin which inhibits secretion of inflammatory molecules from mast cells by affecting moesin, a unique 78 kDa mast cell protein [Theoharides *et al.* *J Pharm Exp Therap* 294:810 (2000)]. In addition to quercetin, other flavones suitable in carrying out the invention include myricetin, genistein and kaempferol.

[020] It should be emphasized that the olive kernel oil, which expression is used interchangeably herein with the expression olive kernel extract, is not olive oil *per se*. Olive oil, which has as its source the flesh of the olive, is discarded in step (1) of the procedure described below. The material used in this invention is an extract derived from the kernel (pit) of the olive.

[021] The olive kernel oil component of the inventive compositions is preferably an unrefined (first pressing, filtered, oleic acid-related acidity <1%, water content <5%) extract produced, for one source, on the island of Crete in Greece. This kernel oil is especially prepared by the maker by a process

consisting essentially of: (1) washing the kernel mass that remains after the compression of the oil from the olive flesh with water; (2) drying the washed kernels in a stream of hot air; (3) extracting the dried kernels with hexane and steam; (4) microfiltering the extract (1 micron pore size) to remove particulate matter; (5) heating the extract at 86-100 degrees while percolating helium (to avoid oxidation) through the fluid to evaporate the hexane (final <1%), which process reduces the water content to <1% and the acidity as oleic acid of <3%.

[022] This olive kernel extract surprisingly has the unique property of increasing absorption of the other components of the anti-inflammatory compositions through the intestinal mucosa or skin (cf., Example 17 below), and also adds its own content of important anti-oxidants [Bosku, *World Rev Nutr Diet*, 87:56 (2000)], such as omega fatty acids (e.g., eicosapentenoic acid) and alpha tocopherol. Although not claimed herein, it has been claimed that kernel olive extract has cytoprotective, longevity-producing effects [Trichopoulou *et al. Am J Clin Nutr* 61:1346S (1995); Trichopoulou *et al, Cancer Epid Biomarker Prevention* 9:869 (2000)]. The polyphenols in such kernel extract also have anti-inflammatory effects in, for example, arthritis [Martinez-Dominguez *et al., Inflamm. Res.* 50:102 (2001)]. A preferred source of the unrefined kernel olive extract of the invention is: E.B.E.K., Inc., Commercial, Industrial Enterprises of Crete, 118 Ethnikis Antistasecos, Heraklion, Crete, 71306, Greece.

[023] Supplementation of the compositions described above with the methylation reagent S-adenosylmethionine ("SAM") adds antioxidant, anti-inflammatory and cytoprotective properties, particularly in inflammatory joint diseases. Addition of SAM also accelerates metabolism of homocysteine, which amino acid has been implicated in coronary disease, to cysteine, which is harmless. Folic acid may be added to certain of the present formulations for similar reasons.

[024] In a highly preferred embodiment, the sulfated proteoglycan is non-bovine chondroitin sulfate, preferably from shark cartilage, which blocks mast cell activation, degranulation and consequent secretion of inflammatory biochemicals from the mast cells. As noted above, other natural sulfated proteoglycans suitable for practicing this invention include keratan sulfate, dermatan sulfate and hyaluronic acid salt, e.g., sodium hyaluronate.

[025] The highly preferred flavone is quercetin which inhibits secretion of inflammatory molecules from mast cells by affecting moesin, a unique 78 kDa mast cell protein [Theoharides *et al. J Pharm Exp Therap* 294:810 (2000)]. In addition to quercetin, other flavones suitable in carrying out the invention include myricetin, genistein and kaempferol. One preferred source of quercetin is the *Saphora* plant or rutin (quercetin glycoside).

[026] Another supplement to the basic compositions of the invention is a histamine-1 receptor antagonist, such as diphenhydramine, hydroxyzine, azelastine, azatadine and ciproheptadine. Other histamine-1 receptor antagonists are described in Table 25-1 in Goodman and Gilman's *The Pharmaceutical Basis of Therapeutics*, 9<sup>th</sup> ed., New York, 1996. Histamine -3 receptor agonists are described in the Theoharides patents listed above.

[027] Inhibitors of mast cell activation and secretion may be used in the treatment of inflammatory processes such as superficial vasodilator syndrome, e.g., menopausal-associated flush, monosodium glutamate-associated flush, carcinoid flush and niacin-associated flush.

[028] Sources of CRH antagonists include, in addition to the Theoharides patents listed in the Background section above: Neurocrine Biochem. Inc.'s D-Phe 12 Nle Ala<sup>32,21,38</sup>hCRH(12-41)NH<sub>2</sub>, cat no. 1P-36-41; Pfizer non-peptide CP-154,526-1; Sigma Chem., St. Louis anti-CRH polyclonal antiserum; and Pfizer, NY patents and applications: US6,211,195, US 5,795,905, PCT/IB95/00573, PCT/IB95/00439, US08/448,539, US 08/481,413, US09/735,841,



and in Owens *et al. Pharm. R* v. 43:425 (1991).

[029] The preferred concentration range of the proteoglycan, hexosamine sulfate and flavone components of the oral formulations are 10-3,000 mg per tablet or capsule. The preferred concentration range for SAM is 3-1,000 mg per capsule or tablet. The preferred concentration range of bitter willow extract is 10-50 mg per tablet or capsule. Generally, where present, the amounts of the olive kernel extract are at least three times those of the other active ingredients, preferably 900-1200 mg. Polyunsaturated fatty acids, preferably from fish oils, e.g., eicosapentenoic acid, may be also present, preferably in amounts similar to that of the olive kernel extract, e.g., 100-300 mg per capsule or tablet. The number of capsules or tablets to be taken per day is determined by the nature and severity of the medical condition, and is readily determinable by the patient's health care provider. Other representative formulations are described in the examples below.

[030] The compositions of the invention may be formulated in any standard means of introducing pharmaceuticals into a patient, e.g., by means of tablets or capsules. The compositions of the invention include ointments and creams for skin conditions, mouth washes and toothpaste for periodontal diseases, and solutions for nasal aerosols. Standard excipients and carriers, e.g., beeswax and lecithin of about 25-100 mg per unit dose, for the active ingredients of the inventive compositions are described in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

[031] Although not bound by any particular mechanism of action of the components of the claimed compositions, the inventor contemplates that the proteoglycan may inhibit the activation and degranulation of the relevant mast cells, while the flavone may inhibit the secretion of inflammatory biomolecules from these mast cells. "Activation" and "degranulation" of mast cells are defined herein as is standard and well known in this art, that is, to mean secretion from the activated mast cell of any type of molecule(s) that alone or

in combination triggers inflammatory processes.

## EXAMPLES

### Example 1

Table 1 compares chondroitin sulfate-containing commercial products to the present compositions.

Table 1

Comparison of commercial chondroitin sulfate-containing products to present embodiments		
Product	Available compositions	Present invention
Main ingredient	Mixture of bovine chondroitins	Non-bovine chondroitin sulfate, preferably the C type
Source	Cow trachea	Shark cartilage
Amount/cap or tab.	100-300 mg	10-3000 mg
Degree of sulfation	Zero to low	High
Target	Unspecified	Mast cells, inflammatory cells
Other ingredients	Vitamins, fish oils	Flavones, unrefined kernel olive oil, SAM, histamine-1 receptor antagonists, histamine-3 receptor agonists, CRH antagonists, polyamines, caffeine, folic acid

<b>Advantages</b>	<b>None known</b>	<b>Anti-allergic, anti-inflammatory, anti-oxidant, cytoprotective</b>
<b>Adverse effects</b>	<b>Risk of mad cow disease, spongiform encephalopathy, stomach upset, allergy to fish products</b>	<b>None known</b>
<b>Relevant conditions</b>	<b>Osteoarthritis</b>	<b>Allergic inflammation angina, asthma coronary artery disease, arthritis (osteoarthritis or rheumatoid arthritis), chronic prostatitis, eczema, fibromyalgia, interstitial cystitis, irritable bowel syndrome, inflammatory bowel disease, migraines, multiple sclerosis, psoriasis, periodontal disease, flush syndrome, cancer (including hormonally-dependent forms).</b>

Scientific publications	None found	Theoharides <i>et al. Br J Pharm</i> 131:1039 ( 2000) Middleton <i>et al. Pharm Rev</i> 52:673 ( 2000)
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In all examples that follow, chondroitin sulfate is of a non-bovine variety.

### Example 2

#### Composition For Protecting Against Inflammatory Diseases)

Two capsules to be taken orally 2-3 times daily, at least one hour before meals

Ingredients, per capsule, mg:

* Chondroitin sulfate	150-300
* OKE	900-1200
* D-Glucosamine sulfate	150-300
* Quercetin	150-300

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### Example 3

#### Composition For Protecting Against Arthritis

Ingredients per capsule, mg:

*OKE	900-1200
*D-Glucosamine sulfate	150-300
*Chondroitin sulfate	150-300
*A hyaluronate salt	100-200
*Quercetin	150-300

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### Example 4

#### Topical Composition For Protecting Against Arthritis

Skin ointment or cream. Apply three times per day to affected areas.

<u>Ingredients</u>	<u>% by weight</u>
*OKE	15
*D-glucosamine sulfate	5
*Chondroitin sulfate	5
*A hyaluronate salt	5
*Bitter willow bark extract	5
*Quercetin	3

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#### Example 5

#### Composition For Protecting Against Cardiovascular Disease

Two capsules to be taken orally 2-3 times per day, in mg:

*OKE	900-1200
*Chondroitin sulfate	50
*Kaempferol	100
*S-adenosylmethionine	50
*Niacin	100
*Bitter willow bark extract, 5% by weight	

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#### Example 6

#### Composition For Protecting Against Periodontal Disease

#### Mouthwash:

*Chondroitin sulfate	0.4 M
*Quercetin	0.4 M
*In a standard mouthwash vehicle	

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#### Example 7

#### Toothpaste Composition

**Toothpaste, mg%:**

\*Chondroitin sulfate 5

\*Quercetin 3

\*Optionally, D-glucosamine sulfate 5

5 \*In a standard toothpaste vehicle

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\* \* \*

**Example 8**

10 **Sunscreen composition**

**Ingredients mg%**

\*Chondroitin sulfate 5

\*D-glucosamine sulfate 5

\*Quercetin 3

15 \*Sun screen (e.g., TiO<sub>2</sub>) 5

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\* \* \*

**Example 9**

20 **Composition For Protecting Against Migraine Headaches**

**Ingredients, mg:**

\*Chondroitin sulfate 50

\*Quercetin 100

\*Azatadine 4

25 \* Optionally, a CRH-receptor antagonist

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\* \* \*

**Example 10**

30 **Composition For Protecting Against Relapsing Multiple Sclerosis**

**Ingredients, mg:**

\*Chondroitin sulfate 50

*Quercetin	400
*Hydroxyzine	50
*Optionally, interferon-beta	

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#### Example 11

#### Composition For Protecting Against Cystitis And Prostatitis

<u>Ingredients,</u>	<u>mg:</u>
*OKE	900-1200
*D-glucosamine sulfate	50
*Chondroitin sulfate	100-300
*Sodium hyaluronate	200
*Quercetin	100-400

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#### Example 12

#### Composition For Protecting Against "Flush"

##### Ingredients, per capsule:

*Chondroitin sulfate	50 mg
*Quercetin	150 mg
*Bitter willow bark extract	5% by weight
*Optionally, cyproheptadine or azatadine	4 mg

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#### Example 13

#### Cream Composition For Protecting Against Skin Allergy

<u>Ingredients:</u>	<u>% by weight</u>
*OKE	15

	*Aloe vera	5
	*Non-bovine chondroitin sulfate	5
	*Myricetin	5
	*Alpha-tocopherol I	5
5	*Optionally, azelastine or hydroxyzine	5

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#### Example 14

#### 10 Composition For Protecting Against Allergy and Allergic Asthma

	<u>Ingredients,</u>	<u>mg</u>
	Myricetin	500
	Chondroitin sulfate	200
15	<u>Optionally, azelastine or hydroxyzine</u>	

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#### Example 15

#### 20 Composition For Protecting Against Hormonally-Dependent Cancers

	<u>Ingredients,</u>	<u>mg</u>
	Quercetin	150
	Genestein	50
	Optionally, tomoxifen or raloxifen	10

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#### Example 16

#### Composition For Protecting Against Allergic Conjunctivitis

	<u>Ingredients:</u>	
30	*Quercetin	0.05%
	* Chondroitin sulfate	2.0%
	<u>*Optionally, azelastine</u>	<u>0.05%</u>



### Figure 17

#### Composition for Protection Against Cardiovascular Inflammatory Disease

5	Ingredients, mg per capsule	
	Chondroitin sulfate	25-75
	OKE	200-300
	Quercetin	50-150
	Rutin	25-75
10	SAM	150-250
	Folic acid	0.17-0.37
	Fish oil polyunsaturated	
	fatty acids	150-200
	Bitter willow extract	10-50
15	Suspension agents	50-70

### Example 18

#### Effect of Olive Kernel Extract on Absorption of Proteoglycan Sulfate *In Vivo*

Chondroitin sulfate was tritiated by New England Nuclear Corp. to a specific activity of 4.3 mCi/ml.

2.5 mCi of tritiated chondroitin sulfate was given orally to 250 g laboratory rats without (control) and with (experimental) OKE. Serum radioactivity was measured 8 hours thereafter.

The results showed that, in control animals, about 3.9% of the dose reached the circulation. In sharp contrast, in animals given OKE along with the labeled chondroitin sulfate, about 14.3% of the dose was absorbed into the general circulation.

**These results demonstrate that OKE increased by almost 400% the absorption of a proteoglycan from the intestine into the general circulation.**